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Antibody response after COVID-19 mRNA vaccination in relation to age, sex, and side effects

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Abstract

Background. The mRNA vaccines for SARS-CoV2 have proven highly effective and are currently used to vaccinate all age groups against COVID-19. Despite their high efficacy in clinical trials, there is limited data on the impact of age, sex, and side effects on vaccine-induced immune responses.

Methods. We here studied the development of SARS-CoV-2 Spike protein RBD domain antibodies after two doses of the Pfizer-BioNTech Comirnaty mRNA vaccine in 118 healthy volunteers and correlated their immune response with age, sex, and side effects reported after the vaccinations.

Findings. Our findings show a robust immune response to the Spike protein's RBD region after the first and the second vaccination dose. However, we also saw a decline of antibody levels at 6 weeks versus 1 week after the second dose, suggesting a waning of the immune response over time. Regardless of this, the antibody levels at 6 weeks after the second dose remained significantly higher than before the vaccination, after the first dose, or in COVID-19 convalescent individuals. We found a decreased vaccination efficacy but fewer adverse events in older individuals, and that mRNA vaccination is less efficient in older males whereas the detrimental impact of age on vaccination outcome is abolished in females at 6 weeks after the second dose.

Interpretation. The Pfizer-BioNTech Comirnaty mRNA vaccine induces a strong immune response after two doses of vaccination but older individuals develop fewer side effects and decreased antibody levels at 6 weeks. The waning of anti-viral antibodies in particular in older male individuals suggests that both age and male sex act as risk factors in the immune response to the SARS-CoV-2 mRNA vaccine.

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Research in context

Evidence before this study

The first studies addressing the immune responses in older individuals after the single-dose administration of the SARS-CoV-2 mRNA vaccines have been published. We searched PubMed and medRxiv for publications on the immune response of SARS-CoV-2-mRNA vaccines, published in English, using the search terms "SARS-CoV-2", "COVID-19", "vaccine response", "mRNA vaccine", up to April 15th, 2021. To date, most mRNA vaccine response studies have not been peer-reviewed, and data on the role of age, sex and side effects on SARS-CoV-2-mRNA vaccines in real vaccination situations is limited. Some studies have found a weaker immune response in older individuals after the first dose and these have been measured at a relatively short period (within 1-2 weeks) after the first dose but little longer-term evidence exists on the postvaccination antibody persistence. Even less information is available on sex differences or correlations with mRNA vaccine side effects.

Added value of this study

In this study, we assessed the antibody response up to 6 weeks after the second dose of Pfizer-BioNTech Comirnaty mRNA vaccine in 118 individuals. Our findings show a strong initial immune response after the first dose and an even higher Spike RBD antibody levels at 1 week after the second dose, but these significantly declined at 6 weeks after the second dose. We also found a weaker immune response and faster waning of antibodies in older vaccinated individuals, which correlated with fewer side effects at the time of vaccinations. Furthermore, although overall female and male vaccinees responded similarly, we found that age-related waning of the vaccine-related antibodies was stronger amongst older males whereas in females the impact of age was lost at 6 weeks after the second dose.

Implications of all the available evidence

New mRNA vaccines are now applied worldwide as they have shown high efficacy in clinical trials. Our results show that two doses of Pfizer-BioNTech Comirnaty mRNA vaccine induce a strong antibody response to Spike RBD region but these high levels decline 1.5 months after the second dose in most of the vaccinated individuals. Nevertheless, even at 6 weeks after the second dose, they stay significantly higher than at prevaccination, after the first dose of vaccine, or in Covid-19 postinfection. These findings also implicate that fewer adverse effects may indicate lower antibody response after the vaccination and point to the need for more individualized vaccination protocols, in particular among older people.

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Introduction

New mRNA vaccines have shown high efficacy in clinical trials and are applied worldwide to millions of people. The first two-dose COVID-19 mRNA vaccine, Pfizer-BioNTech Comirnaty (BNT162b2), accepted for emergency use, was found safe and demonstrated 95% efficacy in phase 3 trials. However, so far exists little data about the extent and duration of the antibody responses after the mRNA vaccination, as well as about the factors influencing the efficacy and side effects in real vaccination situations.

Although highly efficient in triggering immune responses in clinical trials and initial real situation vaccinations, the first published studies with Pfizer-BioNTech mRNA vaccines have reported weaker immune responses and a higher number of non-responders among older people after the single[1,2] and second dose with BNT162b2 vaccine [3,4]. Nevertheless, one study failed to show a significant correlation between age and antibody response after the second vaccination but found a lower magnitude of memory B cell responses with increased age[5], highlighting a need for further studies to understand the age-related responses to mRNA vaccination. Also, limited information is available about the side effects and their correlation with vaccination outcomes. For example, Goel et al[5] found no significant association between the antibody levels and severity of adverse events among vaccinees. Furthermore, the initial studies have not found sex differences in response to COVID-19 vaccination[1,5] which has been widely described with several other vaccines[6].Here we addressed the dynamics of anti-S-RBD IgG vaccination response after first and second doses of the Pfizer-BioNTech Comirnaty mRNA vaccine in healthy volunteers and assessed its correlation with the age, sex, and severity of vaccine side effects.

Material and Methods

Recruitment, sample, and data collection. SYNLAB Estonia employees volunteering to be vaccinated with COVID-19 mRNA Comirnaty (Pfizer-BioNTech) vaccine were invited to participate in the study. Participants signed an informed consent form agreeing with sampling and usage of their clinical data. The blood samplings were performed by trained medical personnel at SYNLAB Estonia. Samples were taken before the first dose of vaccine (baseline), just before the second dose, and 3 weeks after the first dose (time-point 1), 1 week after second dose (time-point 2), and 6 weeks after the second dose (time-point 3). The study participants filled in a questionnaire about the presence of side-effects after the second dose and rated their side-effect severity with scoring from 0 to 3 (Supplementary Table 1). All samples and volunteers' data (age, sex, side effects) were stored in a pseudonymized manner.

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As controls, we used samples from uninfected and non-vaccinated negative controls (n=50) and PCR-positive mild COVID-19 (n=97) patients collected and described previously.[7]

The study has been approved by the Research Ethics Committee of the University of Tartu on February 15, 2021 (nr 335/T-21). Patients signed informed consent before recruitment into the study.

Antibody testing. Serum samples were analyzed for the antibodies to SARS-CoV-2 Spike protein receptor-binding domain (S-RBD) IgG using quantitative SARS-CoV-2 IgG QN chemiluminescent micro-particle immunoassay (CLIA) on ARCHITECT i2000SR analyzer (Abbott Laboratories). The cut-off and the upper detection limit of the test were 50 and 80,000 AU/mL, respectively.

Statistics. The t-test and Pearson correlation calculations were used for data analysis using PAST 4.05 software. Python's (version 3.7) sklearn principal component tool was used to differentiate between younger (40 years and less) and older age groups given the scores of side effects, IgG values and sex as model inputs. The boxplots were visualized using pyplot from Python's matplolib library.

Results

Altogether we studied 118 individuals (21 males and 97 females) who received their first and second COVID-19 Pfizer-BioNTech vaccine doses and gave corresponding pre- and post-vaccination blood samples. From these, 74 individuals were sampled at all post-vaccination time points and 90 completed the questionnaire on post-vaccination side effects. The age of the vaccinated volunteers ranged from 21 to 68 years (median 34).

Antibody dynamics. Three weeks after the first vaccine dose, we found elevated S-RBD IgG levels in vaccinated serum samples (**Figure 1**), measured by the Abbot Laboratories CLIA method, with mean IgG levels of 2,079 AU/mL (range 117 – 9,164). Importantly, these S-RBD IgG levels increased significantly after the second vaccination dose (at 1 and 6 weeks) as compared to the first dose ($p<0.0001$). One week after the second dose, the serum antibody levels were boosted in participants to a mean level of 26,941 AU/mL (range 3,954 - >80,000). However, we found that the S-RBD IgG levels were significantly decreased to 13,971 AU/mL (2,049 – 39,789) ($p<0.0001$) at 6 weeks after the second dose as compared with 1 week after the second dose (**Figure 1**). This trend of declining antibody levels between 1 and 6 weeks after the second dose was present in most of the vaccinees, and on average S-RBD IgG levels decreased 45% between these two time-points. In contrast, we found increased S-RBD IgG levels at 6 weeks after the second dose in only 4% of individuals. One individual had slightly elevated S-RBD IgG before vaccination, but negative anti-Nucleocapsid IgG and anti-Spike IgM, also post-vaccination S-RBD IgG was close to average. Although COVID-19 was never

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diagnosed in this person we could not exclude possible earlier exposure to SARS-CoV-2. We also saw a greater S-RBD IgG decrease in individuals with higher IgG values a week after the second dose (ρ= 0.27, p=0.02) (**Supplementary Figure 1**).

We also compared the post-vaccination results with S-RBD antibodies in COVID-19 recovered patients (**Figure 1**). The post-infection IgG levels (mean 3,932) were somewhat higher than in vaccinated persons who received the first dose (p=0.01) but were significantly lower than in those who received two vaccine doses (p<0.0001).

Factors influencing the vaccination response. The age of vaccinated individuals had a significant negative correlation with S-RBD IgG response in all measured time points. This was the strongest after the first dose (r= -0.45, p<0.0001), but less significant, although still detectable, at 1 week (r= -0.35, p<0.001) and 6 weeks after the second dose (r= -0.29, p<0.01; **Supplementary Figure 2**). We found a similar association while comparing the antibody levels in persons either younger or older than 40 years. The individuals less than 40 years had higher antibody levels after the first dose (means 2,743 vs 1,289; p=0.004), as well as 1 week (30,866 vs 22,706; p=0.066) and 6 weeks after the second dose (15,963 vs 11,698; p=0.04 **Supplementary Figures 3 and 4**).

In a comparison of sex differences, we found a stronger association between age and immune response among males, where the negative correlations between age and S-RBD antibodies were equally strong at all measured post-vaccination time-points - r=-0.58 (after the first dose; p=0.01), r=-0.59 (1 week after the second dose; p=0.01), and r=-0.58 (6 weeks after the second dose; p<0.05). In females, the corresponding correlations were weaker and also declined in time r= -0.35 (p<0.001), r=-0.28 (p=0.01), and r=-0.21 (ns), suggesting that age together with sex-specific factors affect the vaccination outcomes of mRNA vaccines.

Side effects of mRNA vaccination. Vaccination side-effects merit investigation as they are common reasons for vaccine hesitancy. Altogether 93% of participants reported some type of adverse effects. The most common side effects were reported pain or swelling (in 84%) at the injection site, fatigue (64%), malaise (50%), headache (42%), chills (41%), fever, and myalgia (both 34%). The majority of the side effects were present as mild to moderate. However, 20 (22%) persons reported one or several symptoms to significantly disturb his/her daily living activities, and lasting for several days and/or causing absence from work. The total score of side effects (sum of all self-rated side effect scores per patient) ranged between 0 and 27 (mean 7.76). The detailed data on individuals' side effects are presented in **Supplementary Table 1**.

Factors influencing the vaccine side effects. We found several side effects to positively correlate with the immune response to S-RBD. This was seen with the total score of adverse effects, which

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significantly associated with the IgG levels at all time points i.e. after the first dose ($r= 0.3$, $p<0.01$), and 1 week ($r= 0.47$, $p<0.0001$) and 6 weeks after the second dose ($r= 0.46$, $p<0.0001$). An even stronger correlation was present with fever at all time-points: $r= 0.36$ ($p<0.001$), $r= 0.47$ ($p<0.0001$), $r= 0.51$($p<0.0001$), and also other adverse symptoms such as headache, fatigue, malaise, chills, and nausea were positively correlated with the vaccine response (**Supplementary Figure 2**).

The age of vaccinated individuals negatively correlated with the total score of side effects ($r= -0.35$, $p<0.001$) as well as with several specific side effects (**Supplementary Figure 2**). Similar to the overall vaccination response, when comparing the two sexes separately, we again found males to have a stronger correlation of vaccine side effects with S-RBD antibody levels. The associations between the score of all side effects and IgG levels were strikingly higher in males than in females at 1 week after the second dose $r= 0.87$ ($p<0.0005$) vs $r= 0.44$ ($p<0.0001$) and at 6 weeks after the second dose $r= 0.72$ ($p<0.01$) vs $r= 0.44$ ($p<0.001$). Together the results indicate age- and sex-related variations in side effects to mRNA vaccination responses.

Discussion

We report S-RBD IgG responses after COVID-19 mRNA vaccination showing a significant increase in antibody levels after the second dose followed by a 45% decrease during the next 5 weeks. We found significantly higher IgG levels in vaccinees after 2 doses compared to patients recovered from COVID-19.

We found a negative correlation between antibody responses and the age of vaccinated individuals. Age is an important factor that influences vaccine responses, and elderly people have been reported to be poor responders to influenza, hepatitis A and B, and pneumococcal vaccines by developing lower antibody levels and weaker cell-mediated responses.[8–13]. In addition to diminished post-vaccine responses, older individuals tend to have a more rapid waning of antibodies after the vaccinations. The adverse effect of age on COVID-19 mRNA vaccination has been found after the first dose in some studies.[1–4] We here show weaker mRNA vaccine response after the first dose and also 1 and 6 weeks after the second dose, confirming the previous results but also show that this difference equalizes and is less significant at 6 weeks after the second dose. Thus, our results indicate the benefit of the second dose for older individuals and its effect to level up the short-term vaccination response in younger and older persons, although the long-term persistence of post-vaccination antibody levels in older populations remains to be studied.

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Interestingly, although we could not see direct sex differences in the vaccine response, we found a stronger negative association of age and S-RBD antibodies among males, indicating sex differences in mRNA vaccination, which has not been reported by other COVID-19 mRNA vaccination studies. The findings across the vaccination studies investigating the effect of sex on immune responses are largely consistent but not uniform with females having higher antibody responses to dengue, hepatitis A and B, inactivated polio, rabies, smallpox, and trivalent inactivated influenza vaccination, with males having higher antibody responses to diphtheria, meningococcal, pneumococcal, and tetanus vaccination.[6] Our findings show that age remains an important negative factor for mRNA vaccination in males whereas in females the second dose, by and large, abolishes the detrimental impact of age on mRNA vaccination outcome. Despite the smaller representation of male participants in our study, this suggests less efficient mRNA vaccination response in older males and highlights the need to stratify vaccine responses among the elderly.

Common systemic side effects reported for COVID-19 mRNA vaccines are fatigue, headache, muscle pain, chills, and fever.[14] In our study, 93% of vaccinated individuals reported some type of side-effects, which is higher than previously reported 66% of vaccinated seronegative persons.[14] In agreement with our results, the side effects among some groups were seen in 100% of participants of the mRNA vaccine phase 1/2 study.[15] An expected, older participants reported fewer or even no side effects as also seen earlier [4], and the presence and score of side effects correlated with S-RBD IgG responses.

Taken together we report a robust vaccine response after two doses of Pfizer-BioNTech Comirnaty (BNT162b2) vaccine with lower responses and fewer side effects in older individuals. We also found that age-related decreased vaccine response persisted in older males even 6 weeks after the second dose whereas this was no longer present in older female vaccines at this time point.

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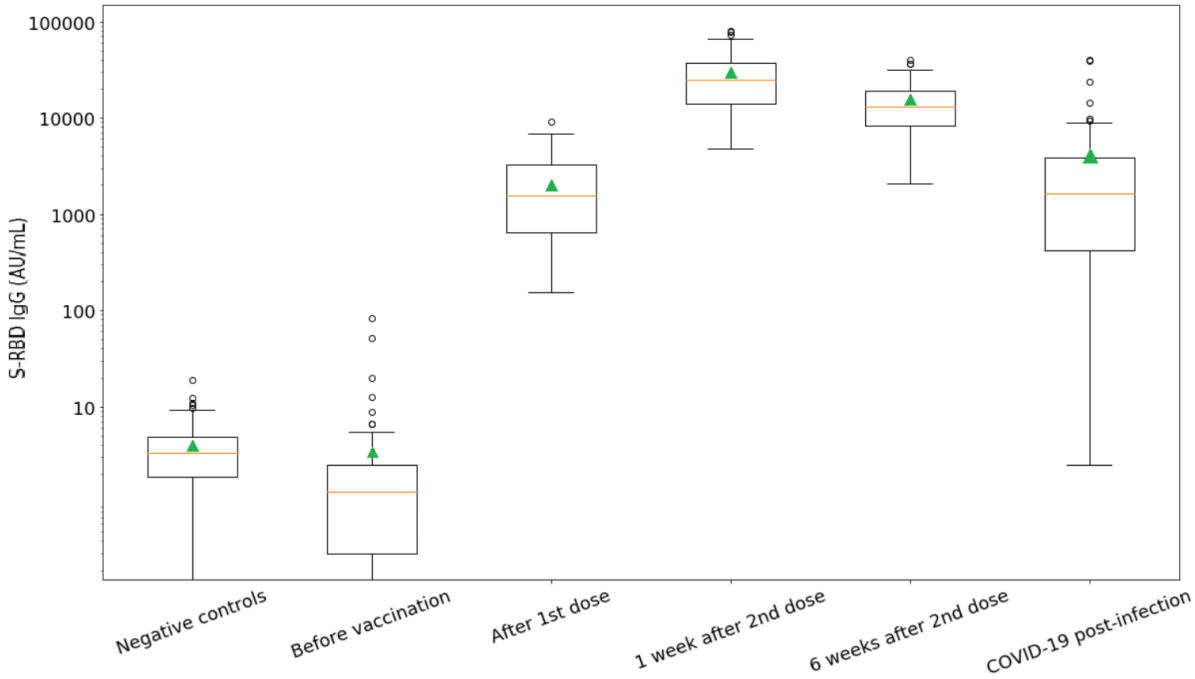

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200 **Figure 1. S-RBD IgG levels before vaccination, after the single and two-dose immunizations with**
201 **Pfizer-BioNTech vaccine compared with pre-COVID-19 negative controls and post-infection levels in**
202 **patients recovered from COVID-19.** The group-wise box-plot comparisons of the S-RBD IgG levels in
203 vaccinated individuals after the first vaccine dose, 1 and 6 weeks after the second vaccine dose, and
204 in COVID-19 convalescent sera. The brown line and green triangle mark median and mean values,
205 respectively.